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AUTOGRAPHA CALIFORNICA NUCLEAR POLYHEDROSIS VIRUS (NPV) IN A VERTEBRATE CELL LINE: LOCALIZATION BY ELECTRON MICROSCOPY

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Abstract.—The nuclear polyhedrosis virus (NPV) of *Autographa californica* has been localized by electron microscopy in the cytoplasm of poikilothermic vertebrate cells (VSW). C-type particles carried by this cell line were also visualized together with baculovirus particles in a vacuole-like organelle. Inoculation of VSW cells with *A. californica* NPV had a retarding effect on cell growth.

Nuclear polyhedrosis viruses (NPVs) are the agents of choice for the control of agricultural and forests pests (WHO 1973). Their selection is based on their specificity for insect hosts and their apparent innocuous nature for higher animals (Heimpel 1966; Heimpel and Buchanan, 1967; Ignoffo and Heimpel 1965). However, it is important that both *in vivo* and *in vitro* systems become available for the evaluation of such agents prior to widespread use in the field. There have been no reports concerning the successful replication of NPVs in vertebrate cells other than that of Himeno et al. 1967. Amongst the theories purported to explain this is that virus does not penetrate vertebrate cells. In the present report we tested this premise. A vertebrate viper cell line (VSW) known to replicate an insect Chilo irridescent virus (McIntosh and Kimura, 1974) was inoculated with *A. californica* AC NPV (Vail et al. 1970) at a multiplicity of infection of 1.5 ($TCID_{50}$/cell). Cultures were incubated at 28°C for 5 days, then prepared for electron microscopy as described previously (McIntosh and Kimura 1974).

The results of the electron microscopy study are present in Figs. 1 and 2. NPV particles (arrows) can be readily seen intracellularly amongst the C-type particles which are carried by this line (Fig. 1). In Fig. 2 a NPV particle with envelope partially removed can be seen extracellularly with many C-type virions. No increase in virus titer could be observed over the 5-day incubation period and this confirms our previous finding (McIntosh and Shamy 1975). However, it was observed that many more C-type particles were present in inoculated cultures than in unchallenged cultures. Since C-type particles do not produce a cytopathic effect, it is not possible to titrate them until such a system is made available. Particle counting using the electron microscope would be an alternative means of quantitating them.

An interesting result was the marked retardation in cellular growth following challenge with AC NPV. At the end of 1 week incubation cell counts

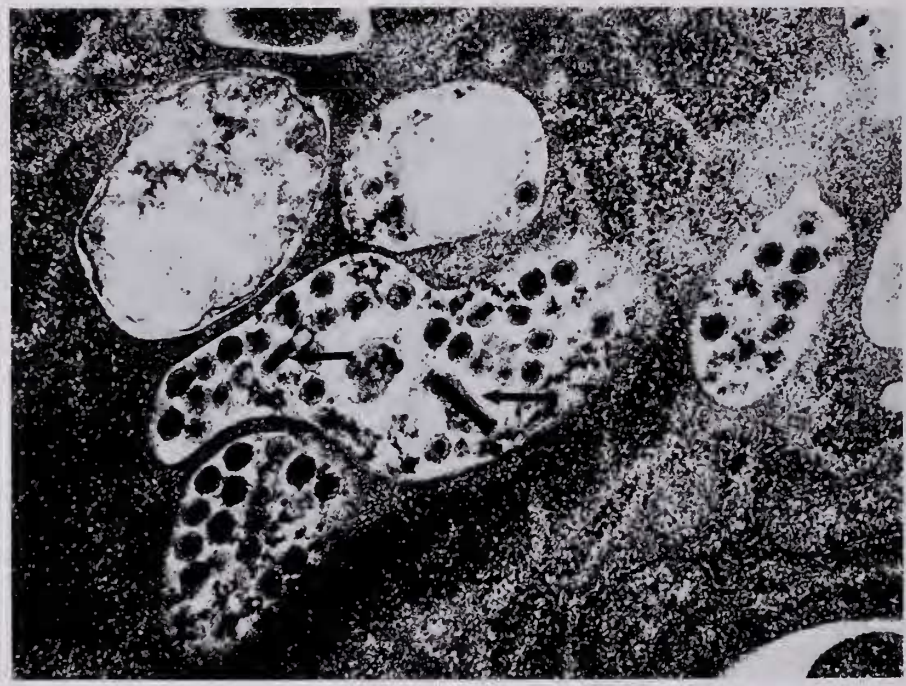

Fig. 1. Intracellular localization of *A. californica* NPV particles (arrows) in a vertebrate VSW cell. Note the presence of C-type particles. ×60,000.

were twice as high in control cultures as in inoculated cultures. When subcultures were made by making a 1:2 split the subculture from the inoculated VSW did not grow out and the pH became very alkaline. Control VSW cells challenged with extracts of the insect cell line TN-368 (Hink 1970) in which AC NPV is propagated, grew normally.

The present findings show that AC NPV is incorporated intracellularly into the vertebrate VSW cell line. The means by which such incorporation occurs are unknown. It is also apparent that intracellular localization of virus particles has a retarding effect on cell growth. It thus appears that failure of AC NPV to replicate in the VSW cell line is not due to lack of uptake by the cells.

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Fig. 2. Extracellular abundance of C-type particles following inoculation of VSW with *A. californica* NPV. A virus particle (arrow) can be seen with partially removed envelope. ×45,000.

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